

A Fresh Look at Metabolic Bone Diseases in Reptiles and Amphibians

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KEYWORDS

- Nutritional secondary hyperparathyroidism
- Renal secondary hyperparathyroidism • Calcium • Phosphorus
- Metabolic bone disease • Reptiles • Amphibians

Metabolic bone diseases (MBDs) are a group of conditions that continue to plague and perplex veterinarians and pet owners. Although the reptile and amphibian (herptile) veterinary knowledge base has grown understanding of the causes and diagnostic and treatment options are often extrapolated from human or other mammalian medicine models. Although the roles of UV-B radiation (280–315 nm wavelength), calcium, phosphorus, and cholecalciferol (vitamin D₃) are better understood in some MBDs, there remain many “X” factors that are not. Likewise, quantitative diagnosis of MBDs has been difficult not only in terms of staging a disease but also regarding whether or not a condition is present. Treatment options also present challenges in corrective husbandry and diet modifications, medication/modality selection, and dosing/regimen parameters.

Green iguanas (*Iguana iguana*) have provided the classic presentations of the most commonly seen MBDs, nutritional secondary hyperparathyroidism (NSHPT) and renal secondary hyperparathyroidism (RSHPT), for many years. Although the popularity of the green iguana as a pet has waned, other herptiles have now filled the void, with MBDs still a common primary presentation to veterinarians for those animals. Lizards and chelonians are the most common groups represented, but snakes, anurans, and urodeles/caudates may also develop and present with MBDs.

With a switch in the common species presenting with MBDs comes new challenges in how to identify and how to correct these diseases. I have seen MBDs in bearded dragons (*Pogona vitticeps*), Asian water dragons (*Physignathus cocincinus*), various chameleon species, leopard geckos (*Eublepharis macularius*), uromastix (*Uromastix* spp), *Testudo* spp, and *Geochelone* spp. Instead of the typical diurnal basking, herbivorous green iguana habitat, the entire spectrum of potential environments and dietary

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options now needs to be addressed for MBDs. Although the classic presentation of rubber jaw and popeye arms and legs is noticeable in some species, in other species there are myriad clinical signs to be aware of.

Radiographs and initial blood work are the standards to start better quantifying the diagnosis, but it is critical to try to apply evidence-based medicine techniques to initially assess, diagnose, treat, and prevent these conditions if veterinary medicine is to advance in addressing them. Anecdotal reports are often helpful as starting points, but peer-reviewed scientific studies will continue to be necessary to support and validate theories, test results, and treatment regimens or to debunk those that are ineffective or even potentially harmful. Where possible, this article references such studies, few though they are at this time. In other cases, well-respected anecdotal herptile veterinary articles or review articles are referenced, with extrapolations directly from human/mammalian studies and personal anecdotal observations referred to at times as necessary.

CALCIUM HOMEOSTASIS

Although each MBD is different, there are some basic concepts that address the physiology behind why these diseases occur. It is critical for this to be understood by veterinarians, veterinary staff, and owners, particularly in terms of future prevention. **Fig. 1** shows these basics facts of calcium/phosphorus/cholecalciferol homeostasis and how when one part goes wrong, the ramifications affect the other parts and even parts outside the standard system. **Fig. 2** is a handout provided by me to all herptile-owning clients, whether or not their pet has an MBD. Readers are welcome to contact me for a copy of this to use with their own clients, as long as appropriate credit is provided and it is not modified.

As a review, calcium is a mineral that needs to be consumed in a herptile's diet (perhaps as part of the environmental water). Calcium is critical for bone development

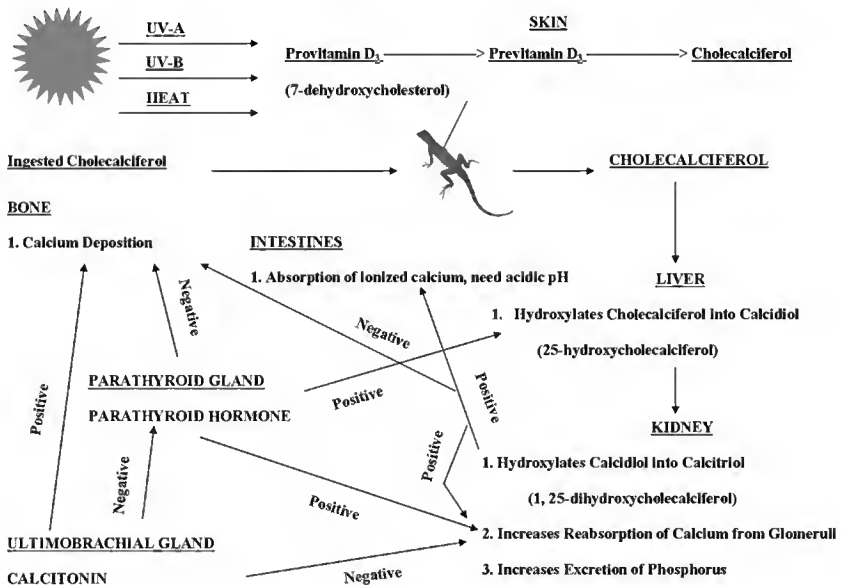


Fig. 1. Calcium and cholecalciferol homeostasis and disease pathophysiology.



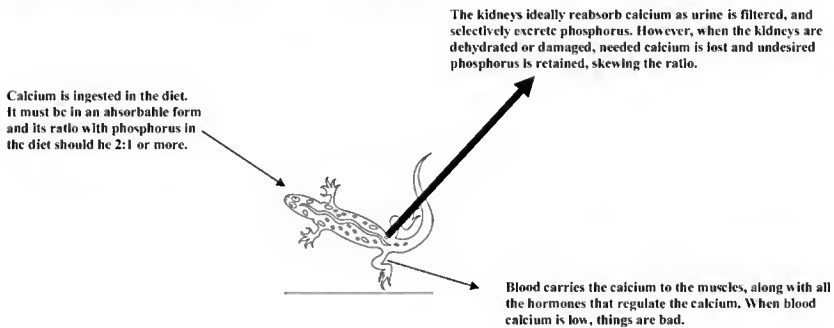
NUTRITIONAL SECONDARY HYPERPARATHYROIDISM Better Known As “METABOLIC BONE DISEASE”

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Nutritional secondary hyperparathyroidism (NSHP) is the most common presentation of metabolic bone disease (MBD) in reptiles & amphibians. MBD encompasses a number of syndromes including NSHP, osteoporosis (bone mass loss), osteomalacia (failure of adult bone to calcify), rickets (juvenile form of osteomalacia), fibrous osteodystrophy (excessive bone resorption and replacement with fibrous tissue), or hypocalcemia (low blood calcium levels).

1. Assume all reptiles & amphibians are either clinical for MBD (showing outward signs) or sub-clinical (it's waiting to happen).
2. We all know that calcium is extremely important to strong bones. But it is probably more important at the cellular level for muscle contractions (especially the heart). It even plays a role in allowing blood to clot. Too much can cause heart attacks and seizures, so there can be too much of a good thing.
3. The third concept is how calcium is taken in, monitored, and maintained. The following diagram may help.

Natural sunlight or an indoor bulb with 2-12% UVB hits the skin, converting the inactive form of vitamin D3 into the active form. This is what determines how much calcium is absorbed from the intestine. Oral forms of vitamin D3 (cholecalciferol) in inappropriate species can cause excessive uptake of calcium and cause blood vessels and internal organs to be calcified. This is how many rat poisons work. The sunlight or UVB will not work through glass, plastic, fine screening or if the UVB is more than 2 feet from the reptile and amphibians.



***Bone is the calcium warehouse, too much loss leads to rubbery bones that easily break.

MORE ON THE BACK!

Fig. 2. Handout provided to clients to explain MBDs (especially NSHP). (Courtesy of the Animal Medical Center, Bozeman, MT; with permission.)

Anything affecting a part of this diagram can mess everything up. In some cases, this is easy to fix, but in most, recovery is long and drawn out at best. As the old saying goes, "prevention is the best cure." It is important to realize that we do not know the perfect set-up for any species or individuals. Reptile/ amphibian medicine is changing rapidly, thus it is important to keep in touch with your reptile/amphibian veterinarians for the latest information.

How can you prevent this or help correct an already existing problem?

By doing the following:

1. **FEEDING THE RECOMMENDED DIET, IN THE CORRECT PROPORTIONS, AT THE CORRECT TEMPERATURE.** See species specific handouts/get diet instructions directly from veterinarian.
2. **USE THE CORRECT BULBS, WITH CORRECT LEVELS OF UVB, SET UP IN THE RECOMMENDED MANNER, WHEN NATURAL SUNLIGHT CAN NOT BE USED.** UVB levels should be 0-12%, dependent on species (ask vet if uncertain) within 2 feet of the animal, with no barriers between, & should be changed every 6 months. Use ZooMed®'s T-Rex®'s fluorescent bulbs or their mercury vapor lamps.
3. **SOAK YOUR REPTILE ONCE DAILY FOR 15 MINUTES IN A SHALLOW, WARM WATER BATH.** Certain species may not have this recommended for them. Keep the temperature at what a small child should be bathed at, and the water should be only one half the height of the reptile, less if they are sick or weak. This helps keep them hydrated, and their kidneys at their peak level of function.
4. **DO NOT USE ORAL VITAMINS CONTAINING VITAMIN D3 OR PHOSPHORUS. DO NOT USE THE SUN SPRAYS OR MOON SPRAYS.** Pet stores often sell products in ignorance, if there is a new product you are interested in, ask your veterinarian. I like to use Tums® ground up into a powder as a calcium source.
5. **DO NOT FEED ANY ANIMAL-BASED PROTEINS SUCH AS MONKEY CHOW/ BISCUITS, DOG FOOD, CAT FOOD, OR OTHER MEATS TO VEGETARIAN REPTILES. FEED HIGH QUALITY PROTEIN TO THE CARNIVORES.**

****These make our reptiles/amphibians real big, real fast, but it damages their kidneys, and severely shortens their lifespans. Remember this when purchasing an older animal, it might have occurred in its past. They like the taste of these products, "but just cuz it tastes good don't mean it's good for 'em!"**

What are the signs to look for in your reptile or amphibian?

1. fractured legs
2. inability to stand upright
3. paralysis
4. swollen "Popeye" arms and legs
5. rubber jaw, upper jaw longer than swollen lower jaw
6. finger, toe, tongue, and pupil tremors
7. stunted growth
8. unable to eat, though hungry
9. abnormal shell growth in turtles and tortoises
10. scoliosis of the back (S-curved)
11. inability to urinate and defecate
12. weakness and collapse
13. DEATH

All of these are signs which indicate your reptile/amphibian needs to be seen by your veterinarian. There are some diagnostic tests that can determine severity of the MBD, secondary complications (dehydration, infection, liver disease), and the best course of action. Likewise there are treatment regimes that can buy more time or even correct the situation. MBD TAKES A LONG TIME TO DEVELOP & A LONG TIME TO CORRECT IN THOSE SITUATIONS WHERE CORRECTION IS POSSIBLE!

Fig. 2. (continued)

and maintenance, egg production, and cellular/muscle activity, with the last including the heart, the gastrointestinal tract (GIT), and the brain. Calcium comes in several forms and, as discussed later, the form plays a significant role in how much calcium is absorbed and how it is used. Calcium absorption is regulated by the type of calcium, levels of phosphorus in the diet, the health of the GIT, cholecalciferol (vitamin D₃) levels, calcitonin levels, and PTH levels. Calcium and phosphorus form a conglomeration, called hydroxyapatite, which is the main component of bone and teeth. As in mammals, magnesium, among other factors, may also play roles in calcium regulation; however, supportive evidence is lacking for roles or lack thereof at this time.

The next factor to consider is cholecalciferol. How this works depends on the type of herptile. For most snakes,¹ crocodilians, some chelonians, and some amphibians, life-style (nocturnal) and ingestion of whole prey seem to have led to an evolution whereby cholecalciferol is completely (or nearly completely) collected orally from the diet (see later discussion on UV-B). For many commonly kept lizards that are diurnal and

insectivorous or herbivorous, some chelonians, and some amphibians, UV-B (280–315 nm wavelength) and UV-A (315–400 nm wavelength) are necessary to activate the cholecalciferol pathway (see [Fig. 1](#)). Provitamin D₃ (7-dehydrocholesterol) in the skin is converted by UV-B and UV-A to previtamin D₃. Previtamin D₃ is thermochemically isomerized into cholecalciferol in the skin, with higher temperature increasing the rate of isomerization; however, if excessive levels of cholecalciferol develop, the same process can break it down into inert tachysterol or lumisterol or back into provitamin D₃.² It is undetermined whether or not this reversal mechanism works in herptiles relying completely on dietary cholecalciferol, particularly in the case of ingestion of toxic levels; however, it can be surmised that the system is built to reduce mild or slowly increasing excesses, not a sudden rush.

Once cholecalciferol is inside the body, whether or not from diet or from UV-B/UV-A conversion, the next steps are the same. Cholecalciferol travels to the liver, where it is hydroxylated into calcidiol (25-hydroxycholecalciferol), then travels to the kidney for a final hydroxylation to become calcitriol (1,25-dihydroxycholecalciferol)—the biologically active form of vitamin D₃.

Calcitriol is what increases calcium and phosphorus absorption from the GIT, increases bone release of calcium (demineralization), induces immune system stimulation (in mammals at least), and creates a relatively inhospitable microenvironment for neoplastic cells (in mammals at least).² In certain situations, calcitriol is regulated by estrogen, prolactin, and growth hormone in mammals; although regulation is likely similar in herptiles, further research needs to be done to confirm and perhaps elucidate the presence of other factors influencing calcitriol levels and effects.² Current levels of calcium and phosphorus, among other parameters, can affect renal hydroxylation of calcidiol. Parathyroid hormone (PTH) release promotes increased renal hydroxylation, so that more calcitriol is produced. In opposition, increased levels of calcitonin from the ultimobranchial glands inhibit demineralization of bone (and thus enhance remineralization) and have a negative feedback directly on PTH release. Although calcitonin evolved back at least as far as fish, PTH likely evolved in amphibians and was retained further up the evolutionary tree.² Although PTH has several redundant effects duplicated by other organs or hormones, from an evolutionary perspective, there developed a need for that redundancy.

Bone serves as a repository, releasing or storing calcium and phosphorus as directed by calcitriol, calcitonin, or PTH, thus filling a passive role. The kidneys are the final piece of the puzzle. They play a hydroxylation role in the final steps of converting calcidiol. They also respond to calcitriol to increase calcium reabsorption in the glomerulus and increase phosphorus secretion. Effects of PTH and calcitonin can affect hydroxylation activity in the kidney and, therefore, indirectly affect calcium homeostasis (discussed previously).

TYPES OF METABOLIC BONE DISEASES

There are many types of MBDs and, although a layperson may use the singular term, MBD, veterinarians must first identify which type of MBD exists in terms of: diagnostics, treatment, ramifications, prevention, and prognosis. There is an excellent summary of MBDs by Mader in *Reptile Medicine and Surgery*.³ Many pictures and diagrams in the chapter demonstrate the clinical presentations of the disease, and the author strongly encourage readers to take advantage of the information provided in Dr Mader's chapter.

The two most commonly presented and identified types of MBDs are NSHPT and RSHPT. Most MBDs present to a veterinarian late in the disease process due to

a combination of owner ignorance, animal stoicism, and slow progression. This is an important concept to remember when presenting initial findings and recommendations to a client for the first time. This is also relevant when discussing prognosis, diagnostics, treatment, and prevention; the latter three are particularly concerning in terms of financial and emotional costs.

NSHPT presents in most cases as a juvenile or immature herptile. Owners are usually new to owning herptiles in general or, although owning that particular species, or they have lost several herptiles over a period of time and have self-diagnosed MBD or just want to stop losing these animals. Most are quite progressed by the time an owner realizes a problem (or decides to address a problem) or even when a veterinarian recognizes a problem. In these cases, inadequate oral calcium, a poor calcium/phosphorus ratio, inadequate oral cholecalciferol/UV light, inappropriate heating/humidity, or often a combination of several of these factors leads to a physiologic response to depleted calcium levels by increasing PTH production. Because needs are not met, calcium depletion from bone becomes clinical and the overall shortages start affecting muscle calcium needs.

In most lizards, there may be a history of several individuals housed in the same cage or in close proximity. This stress seems to predispose to a variety of secondary diseases, including NSHPT. Bearded dragons tend to develop better individually when housed in confined spaces. Additionally, bearded dragons not allowed to bask in ideal areas for heat and UV light and forced to eat undesired leftovers in food bowl have compounded stressors. They often lay down flat most of the time and may have a rubber jaw with lip deformations and secondary chelitis/gingivitis/stomatitis and intention tremors in the extremities and tongue. These pets want to eat but select soft foods if they eat at all. Prehension may be slowed or may not occur, thus drawing out the eating process. Constipation affects many species in all types of MBDs. With calcium's effect on GIT motility, cloacal prolapse or diarrhea may also be presenting complaints.^{3,4} Pathologic fractures of the long bones or mandible are common. Temporary paralysis of the caudal limbs acutely or severe spondylosis due to vertebral fracture and callus can be seen. Pelvic fractures and malunions can lead to dystocia or constipation. In chameleons and anurans, an inability to retract their tongues after attempted prey capture has been noted. Chelonians often have very soft, squishy shells, malformed rhamphothecas (beaks), and caudal paresis. In advanced stages, seizures, respiratory/cardiac compromise, and death occur. In my opinion, it is likely that described UV-B deficiency seen in some snake species (discussed later) is a variation of NSHPT, although again that is purely conjecture on at this point.

RSHTPT tends to be a condition of older animals, where a slow decline in kidney function eventually leads to an inability to respond to signals for the kidneys to selectively retain calcium and secrete extra phosphorus. Often gastrointestinal absorption can compensate for this declining function, but eventually the combination of this issue and the inability to hydroxylate calcidiol to calcitriol leads to a nonresponsiveness of the system to PTH and the hypertrophy of the parathyroid glands. Of greater concern seems to be the resultant hyperphosphatemia due to loss of selective secretion of excess phosphorus. Unlike with NSHPT, many of these herptiles have not even begun to tap far into their bone calcium reserves. These animals often present with seizures and collapse or intention tremors occasionally due to acute loss of calcium but more commonly due to hyperphosphatemia. More often than not, RSHTPT herptiles present as anorexic or lethargic. Initial blood work results often indicate the diagnosis. When palpable, kidneys may be swollen, normal, or nonpalpable—potentially indicating chronic renal failure at various stages.

Hypertrophic osteodystrophy has been reported only in lizards. Clinical signs include lameness, painful limbs, and reluctance to move. As with mammals, pulmonary pathology is reported, although with no mention of clinical manifestations; in lizards this condition has an idiopathic etiology.³ In reptiles, osteopetrosis usually manifests as lameness due to bone brittleness and fracturing.³ Osteitis deformans has been described in herptiles; more recent literature suggests that it may actually be a progression of osteomyelitis to a chronic osteoarthritis and spondylosis, particularly in snakes.^{3,5}

Although not reported in herptiles, in theory a parathyroid gland neoplasm (primary hyperparathyroidism) could lead to a calcium imbalance. Likewise, some birds can so deplete their bone reserves from chronic egg production and laying that pathologic fractures as seen in herptiles with NSHPT can occur but again, to my knowledge, this phenomenon has not been reported in herptiles. Reptiles have much less calcium in the shells of their leathery eggs and amphibians have even less.

RAMIFICATIONS

Addressing the presenting symptoms and underlying cause needs to be considered. As in all clinical presentations, make sure to rule in or out other conditions and particularly watch for concurrent issues or secondary opportunistic conditions. These animals often need special nutritional support, potentially for many months. Secondary, opportunistic infections from otherwise benign parasites, bacteria, or fungi can occur. In mammals, most MBDs are considered extremely painful. Unfortunately, pain models in reptiles are only beginning to be developed and, although they exist in anurans at least, in all herptiles there is a challenge to evaluate analgesic options, dosages, and regimens for these stoic animals. Veterinarians need to discuss the ethics, the frustrations, the emotional exhaustion, the quality of life, and often guarded prognoses with clients early in the process and make sure that progression in managing a case is in the patient's best interest, not the client's or veterinarian's. Long-term and short-term prognoses in MBD herptiles are often guarded to poor, owing to the chronicity of disease before diagnosis and treatment plan are made. Likewise, an owner who is told that an initial estimate for diagnostics and treatment will run into the hundreds of dollars for a 25% chance of long-term success, added to hundreds of dollars more for often complete revamping the herptile's environment, often pushes the only feasible options to a choice of euthanasia and hopefully client education to minimize recurrence in future animals.

DIAGNOSTICS

Begin with an extremely thorough history, particularly regarding environment and diet. Physical examination and signalment likewise can fuel suspicions of an MBD. A complete blood cell count may not directly enhance this diagnosis, but concurrent issues often set the table for overall therapy and prognosis. A plasma chemistry panel can be a first step to indications of an MBD. Look particularly at total calcium and phosphorus, their individual values and their relationship. Hypocalcemia, hyperphosphatemia, a less than 1:1 total calcium/phosphorous ratio, and occasionally hypercalcemia (can be high, however, in females and even normal in some species, such as indigo snakes) are initial indicators of potential MBDs. With many animals, lymphatic vessels and sinuses are often in close proximity to blood collection sites, and contamination with significant amounts of lymphatic fluid or pericardial fluid of cardiac phlebotomy may skew those results.

Ionized calcium has been closely studied for its value in diagnosing MBDs in green iguanas.⁶ It is important not to draw conclusions from one herpetile to all others. With few literature reports, however, veterinarians are often forced to do that in practice. Many blood parameters seem to be well conserved across orders and even classes and, if results are interpreted in light of the clinical presentation, decisions and interpretations can be made. In a study evaluating healthy green iguanas, the mean ionized calcium concentration measured in blood was $1.47 (\pm 0.105 \text{ mmol/L})$, with no significant variation by gender or age.⁶ I have seen otherwise healthy immature green iguanas have measured values approaching 2.0 mmol/L on repeated assessments with no apparent clinical manifestations. Challenges with this test include the correct collection/storage of sample and delivery to an outside laboratory. Contacting the primary diagnostic laboratory before sample collection is recommended to confirm correct technique. In mammalian and bird species, portable instant blood evaluation units have been used for faster results.

Several studies have looked at vitamin D₃ levels in the blood or, more specifically, calcidiol (25-hydroxycholecalciferol).^{7–11} Commercially, this test is available through the Michigan State University Diagnostic Center for Population and Animal Health (DCPAH Endocrine Diagnostic Section, PO Box 30076, Lansing, MI 48909-7576; [517] 353-0621; www.animalhealth.msu.edu). It is highly recommended to contact the laboratory before sample collection to determine sample type, shipping requirements, and necessary quantities required. They have panels that also include ionized calcium, PTH, and an option for veterinary endocrinologist interpretation. In red-eared sliders (*Trachemys scripta elegans*), calcidiol concentrations differed significantly between turtles provided supplemental UV radiation ($71.7 \pm 46.9 \text{ nmol/L}$) and those not provided UV radiation ($31.4 \pm 13.2 \text{ nmol/L}$).⁷ A study of 22 healthy, adult tortoises (*Testudo hermanni*, *T. graeca*, and *T. marginata*) of mixed gender kept in captivity under natural unfiltered sunlight in southern England with no dietary sources of cholecalciferol found that the concentration of calcidiol did not vary significantly with the seasons. The concentrations in the female tortoises, however, were significantly lower than in the males.⁸ Calcidiol values for 22 wild Ricord's iguanas (*Cyclura ricordii*), 7 wild rhinoceros iguanas (*C. cornuta cornuta*), and 13 captive rhinoceros iguanas held outdoors were sampled. In a separate study, mean concentrations of calcidiol were 554 nmol/L for wild Ricord's iguanas, 332 nmol/L for wild rhinoceros iguanas, and 317 nmol/L for captive rhinoceros iguanas. On the basis of these results, serum concentrations of at least 325 nmol/L for calcidiol were considered normal for healthy Ricord's and rhinoceros iguanas.¹¹ A group of captive adult Fijian iguanas (*Brachylophus fasciatus*) and (*B. vitiensis*) had their calcidiol status compared with that of agamid and iguanid lizards housed in indoor enclosures under artificial UV light or exposed to natural sunlight (wild-caught or captive animals housed outdoors). Those under artificial lighting had a significantly lower calcidiol status than those housed exclusively outdoors, whereas the calcidiol status of Fijian iguanas that had received intermittent exposure to natural sunlight was intermediate and not significantly different from that of animals housed exclusively outdoors. Captive Fijian iguana eggs, however, had substantially lower calcidiol content compared with eggs from outdoor iguanid and agamid animals. Artificial UV light, therefore, might not be an adequate substitute for natural sunlight to maintain vitamin D status of lizards.¹⁰ Voluntary exposure to higher UV-B irradiance (70 vs $1 \mu\text{W/cm}^2$) resulted in greater circulating calcidiol levels in female panther chameleons (*Furcifer pardalis*) (1510 to 230 nmol/L 604 vs 92 ng/mL).⁹

Radiographs have been used in many cases as a primary diagnostic tool for MBD. Radiographs only detect 30% or greater change in bone density, however, and if total

bone density is poor, then evaluation is often subjective for nonradiology specialists, particularly in immature, small, or reproductively active animals. The advent of digital radiology does seem to have removed a great amount of technique error, although current machine limits (particularly with small specimens), poor positioning, and over-interpretation of findings can affect diagnosis. Again, the author refer readers to Dr Mader's chapter for photographic examples of these conditions.³

A diagnostic tool used in humans to better quantify bone density, particularly in women with osteoporosis, is the dual energy x-ray absorptiometry (DXA) scan, sometimes referred to as a bone density scan. I have used this modality in a RSHPT green iguana to quantify current bone density and monitor therapeutic response. In humans, child values cannot be compared with adult values due to overall bone mass differences. Likewise, as with routine radiographs, most DXA scans are 2-D so cannot account as well for individuals with dense bones. These two limitations may also apply to herptiles. Challenges with this diagnostic tool include the need to position as recommended for humans; it is challenging to account for the tail. This positioning usually requires anesthesia because lack of movement beyond breathing is critical and the scan can take some time depending on an animal's size (scan for a mature male took 30 minutes). A DXA scan generally reads to estimated calcium levels in individual bones, although they can be summed to estimate whole body calcium levels. In the published study using DXA scan, reference bone density values in relation to body weight, gender, and MBC were collected in 28 green iguanas. The regions of interest were the head, lumbar spine, right, and left femur. Body weight had the strongest relationship with bone density. Within regions of interest, for iguanas of average weight (710 g), statistically significant differences between healthy and sick animals were found: head (0.140 vs 0.090 g/cm²), lumbar spine (0.164 vs 0.107 g/cm²), right femur (0.103 vs 0.076 g/cm²), and left femur (0.103 vs 0.078 g/cm²).¹²

Ultrasound as a modality can be used to visualize the kidneys and as a guide for fine-needle aspirates. Use of this modality requires an understanding of kidney location; the kidneys may be more in the pelvic canal versus the abdomen. Kidneys in the pelvic canal will require a more unique approach for visualization.

Glomerular filtration rate was evaluated in 2-year-old healthy green iguanas by IV administration of iohexol (75 mg/kg) into the ventral coccygeal (tail) vein. The mean (SD) glomerular filtration rate was 16.56 mL/kg/h (± 3.90).¹³ This is a helpful value to use for determination of RSHPT.

Another, more advanced diagnostic modality to better evaluate reptilian kidneys is nuclear medicine. A limitation is having the necessary facility and, again, it general requires sedation without movement. Radiopharmaceuticals need to be injected into the bloodstream, as described for two different herptiles (discussed later). Ten healthy green iguanas were evaluated for the determination of kidney morphology and function by the use of scintigraphy involving technetium Tc 99m diethylenetriamine pentaacetic acid (99mTc-DTPA) or technetium Tc 99m dimercaptosuccinic acid (99mTc-DMSA). The researchers found that the use of 99mTc-DTPA for renal scintigraphy was nondiagnostic because of serum protein binding and poor renal uptake of the isotope. Renal uptake of 99mTc-DMSA produced distinct visualization of both kidneys. Renal uptake and soft tissue clearance of 99mTc-DMSA increased over the 20-hour imaging period with a mean renal uptake of 11.31% ($\pm 3.06\%$) at 20 hours. Results indicated that the kidneys of iguanas can be evaluated scintigraphically by use of 99mTc-DMSA.¹⁴ The efficacy of three radiopharmaceuticals, 99mTc-DTPA, 99mTc-DMSA, and 99mTc-mercaptoacetyl triglycine (99mTc-MAG3), for renal imaging was examined in 16 corn snakes (*Elaphe guttata guttata*). All snakes received the radiopharmaceutical via an intracardiac injection. The kidneys could not

be visualized in the three snakes that received ^{99m}Tc -DTPA or in the three snakes that received ^{99m}Tc -DMSA but were well delineated in all 10 snakes receiving ^{99m}Tc -MAG3. These snakes were anesthetized and a dynamic frame mode acquisition was obtained for 30 minutes immediately after injection. A 60-second single static frame mode image was then obtained with the snake in a curled position. Pericardial injections could be a potential contributor to erroneously interpreted results. The mean renal uptake was 25% ($\pm 9.8\%$). Correction for remaining radioactivity in the heart did not seem to be necessary if it was than 10% of the total dose. ^{99m}Tc -MAG3 provided consistently high-quality images of the kidneys and further studies are warranted to evaluate its sensitivity for detecting decreased function in snakes with renal disease.¹⁵

Biopsies are another component of MBDs. The results depend, however, on the quality and location of the bone sample and the experience of pathologists in evaluating herptile samples versus more common mammalian samples. Also, the results need to be considered in conjunction with other findings. Herptile bones seem to be less dense than mammalian bones, so collection technique needs to be appropriate; also, many suspect cases are in animals with pathologic weakening of the bone, so avoiding iatrogenic fractures is critical. With RSHPT, bilateral endoscopic renal evaluation and biopsy can provide tissue samples of excellent diagnostic quality, although proper equipment and training, especially geared toward unique anatomic features of green iguanas, are necessary.¹³ It is assumed that endoscopic renal visualization and biopsy can also be useful in other herptile species.

HUSBANDRY AND DIET FACTORS

Husbandry and diet are two critical areas of management for MBDs and often require point-by-point, in-depth recommendations for clients. These expectations and how nothing will change if they are not addressed must be reiterated with clients, in-person and in discharge handouts. Veterinarians must continue to keep current on those recommendations because they constantly change, often requiring World Wide Web research or discussion with successful herpetologists of a particular species in zoos or the general public. Further research into their natural history and environments also provides insight into specific species' care. Finally, everyone must realize that the requirements to keeping a particular herptile in an ideal captive situation varies significantly by geographic location.

Temperature is an often overlooked factor for ideal reptile health and in particular for the prevention of MBDs. Uptake and action of UV radiation (UV-A and UV-B) needs to occur in a microenvironment that is of the appropriate temperature. Many times, herptiles can self-regulate temperature appropriateness if given a temperature gradient to select from. Many herptiles seem to shun warm areas because of bright light in their eyes or that they feel exposed in the heat and light because of a lack of hiding opportunities. Each species has species-specific temperature ranges. Clients also need to recognize that changes in indoor house temperature due to seasonal variation require the modification of heating set-ups dependent on strategic placement of digital thermometers. For me, in summer, some of my reptiles require supplemental only at night whereas in winter in colder climates, three separate ceramic heat emitters may be needed 24 hours per day to maintain the same temperature. Owners often do not notice this. One option in colder climates is to encourage owners to have summer and winter enclosures, with the latter smaller but easier and more economical to heat.

UV-B radiation exposure, dietary cholecalciferol, and skin-generated vitamin D₃ synthesis were compared between adult males of two species of Jamaican anoles (*Anolis* spp). The more shade-tolerant and thermal-conforming *A lineotopus merope*, rarely exposed to full sun, experienced less UV-B irradiation in its shady environment than the more heliophilic and thermophilic *A sagrei*, which frequently basked in full sun during morning. Both species obtained detectable levels of cholecalciferol in their diet, but the heliophilic *A sagrei* obtained more. To compensate for less availability of UV-B and cholecalciferol, the skin of *A lineotopus merope* seems to have acquired a greater sensitivity than that of *A sagrei* regarding UV-B–induced vitamin D₃ photobiosynthesis, which was assessed by observing a greater conversion of provitamin D₃ to photoproducts in skin exposed to UV-B from a sunlamp. The reduced skin sensitivity of *A sagrei* regarding vitamin D₃ photobiosynthesis may reflect a correlated response associated with less need for vitamin D₃ photobiosynthesis and a greater need for UV-B screening capacity as an adaptation to a more damaging UV-B environment. The possibility, however, that adaptations for photobiosynthesis of vitamin D and for protection from skin damage could involve independent mechanisms needs investigation.¹⁶

There is no substitute for unfiltered, natural sunlight. For several reasons, however, many pet reptiles cannot get adequate exposure to this. And what constitutes adequate exposure? Latitude, time of year, temperature, species, and elevation need to be considered. The right answer is neither obvious nor easy to determine. If clients can take their herptiles outside, they need to watch for overheating, UV burns (usually on the dorsum or eyes—hard to differentiate from thermal burns), and the fact that many herptiles become extremely active and fast moving in natural warm sunlight, leading to a herptile potentially engaging in aggressive or evasive action.

In the laboratory, panther chameleons exhibited a positive phototaxis to greater visible UV-A and UV-B light. With equivalent high irradiances of UV-A or UV-B, however, their response to UV-B was significantly greater than to UV-A. Exposure of in vitro skin patches of panther chameleons to high UV-B (90 $\mu\text{W}/\text{cm}^2$) for 1 hour significantly enhanced cholecalciferol concentration.⁹ Voluntary exposure to higher UV-B irradiance (70 vs 1 $\mu\text{W}/\text{cm}^2$) resulted in greater circulating calcidiol in female panther chameleons (604 vs 92 ng/mL). Depending on dietary intake of cholecalciferol, chameleons adjusted their exposure time to UV-B irradiation as if regulating their endogenous production of the hormone. When dietary intake was low (1–3 IU/g), they exposed themselves to significantly more UV-producing light; when intake was high (9–129 IU/g), they exposed themselves to less. Vitamin D₃ photoregulation seemed to be an additional component of the function of basking.⁹ Panther chameleons with low dietary cholecalciferol intake significantly increased exposure to UV in natural sunlight compared with those with high dietary cholecalciferol intake. All lizards fed low dietary cholecalciferol regulated within optimal UV levels with extreme effectiveness (ability to regulate within optimal UV levels relative to available UV). Chameleons of both dietary treatments regulated UV exposure with great precision, exhibiting little variation among individuals within treatments. This demonstrated the importance of basking for nonthermoregulatory purposes and as an integral mechanism for the regulation of vitamin D₃. Panther chameleons behaviorally regulate exposure to UV in natural sunlight with high precision, accuracy, and effectiveness.¹⁷ A study of red-eared slider turtles post estivation found that the mean calcidiol levels differed significantly between turtles provided supplemental UV radiation (71.7 nmol/L) and those not provided UV radiation (31.4 nmol/L).⁷ As discussed previously, anoles showed selective exposure behavior when exposed themselves to natural UV as needed,¹⁶ and varying UV levels

opportunities should be provided in captivity for herptiles also, as recommended for heat. Places to hide from UV radiation and to partial expose only certain parts of body to UV can allow for better opportunities. Likewise, UV exposure with varying thermal opportunities is important. As with red-eared sliders, juvenile Blanding's turtles (*Emydoidea blandingii*), had significantly higher circulating calcidiol levels when supplemented with UV-B radiation. This leads to the recommendation for UV supplementation for a second basking site, if several semiaquatic chelonians are housed in the same enclosure.¹⁸

What follows is a simplification of how I have interpreted scientific studies and natural history observational reports on species; however, each species is unique and research should be performed on unfamiliar species to see whether or not these rules of thumb are applicable. Opportunities to get out of this intense level of UV-B, as in the wild, are critical to avoid UV radiation and thermal burns.

- For desert-dwelling diurnal lizards or chelonians, high UV-B levels (10% or full unfiltered sun, 12 hours) are recommended.
- For diurnal arboreal lizards or semiaquatic basking chelonians, moderate levels of UV-B (5%, 12 hours) are recommended.
- For diurnal terrestrial lizards or chelonians from a forested environment, low levels of UV-B (5%, 6 hours) are recommended.
- For nocturnal lizards or amphibians, low levels of UV-B (2%, 6 hours) are recommended.
- Snakes they seem to get adequate levels of calcium and cholecalciferol from the ingestion of whole vertebrate prey (or earthworms—usually *Lumbricus terrestris* in the case of many minute snake species or juveniles). An exception to this, anecdotally, may be diamond pythons (*Morelia spilota spilota*), indigo snakes (*Drymarchon corais*), some aquatic species, the insectivorous rough and smooth green snakes (*Ophiodrys aestivus* and *O. vernalis*), and other arboreal, diurnal snakes.² I have had cases of pathologic rib fractures in green tree pythons (*M. viridis*) with minimal handling that anecdotally resolved with the addition of low level UV-B. There are no known studies, however, that have more thoroughly evaluated this urban legend in these snake species.
- There are differences of opinion on the need for UV-B for crocodilians and many chelonians, although it may be best to default to at least minimal to moderate levels of UV-B until studies better determine their needs.
- Albino (amelanistic), hypomelanistic, snow, blizzard, pastel, tangerine, lavender, yellow, pied, anerythristic, leucistic, xanthochromistic, or any other genetic mutant with less than normal levels of melanin are more susceptible to UV light burns of the eyes and dorsal skin, so lower levels of UV supplementation (if any) should be provided with plenty of hiding spots and close monitoring of the skin.

For most captive herptiles, it has been recommended to supplement the diet with calcium of some sort, except for those eating whole prey. Unfortunately, there are few scientific studies evaluating calcium types in herptiles, so medical research is extrapolated from humans with osteoporosis or children with MBDs. In mammals, an acidic intestinal pH is required for calcium absorption. Calcium is also absorbed in the ionized form. How herptiles actually eat their food seems important in how they process or digest the nutrients. Do they swallow the prey whole, or do they chew it? The answer to the question may determine whether or not prey should be gut loaded or dusted and why there are conflicting studies on the benefits of these two techniques. In juvenile leopard tortoises, one group was fed a basic low-calcium feed for 6 months, and the other 3 groups were fed the same basic diet

supplemented with 1, 3, and 9 times the amount of calcium recommended as a supplement to the diet of reptiles. The animals' bone mineral content and bone mineral density were estimated by DXA scan. Those receiving no calcium supplement had bone calcium depletion, and the shell of the tortoises receiving the recommended level of calcium did not calcify to the extent expected. Tortoises receiving 3 times the recommended level of calcium supplementation had the highest growth rate and were thriving. Metastatic calcifications were observed post mortem, however, in the 2 groups that were given the highest doses of calcium.¹⁹

Calcium comes in many forms. Although there is a lot of debate on which form is best, it is important to understand the strengths and weaknesses of each type, at least as they are understood in humans. Calcium carbonate (Tums, coral calcium, or cuttlebones) is the least expensive and should be taken with food. It requires low pH levels for proper absorption in the intestine. In humans, calcium absorption from calcium carbonate is similar to the absorption of milk calcium from milk. Calcium carbonate is 40% elemental calcium (1000 mg = 400 mg of calcium). Calcium citrate does not need to be taken with food, is less likely to cause constipation and gas, and has a lower risk of contributing to kidney stone formation. Calcium citrate is 21% elemental calcium (1000 mg = 210 mg calcium). It is more expensive and more of it must be taken to get the same amount of calcium. Calcium phosphate splits the difference in cost, is easily absorbed, and is the least likely to cause constipation and gas. Calcium lactate has similar absorption as calcium carbonate but is more expensive. Calcium lactate, calcium gluconate, and calcium glucubionate are less concentrated forms of calcium and are not practical oral supplements in humans often due to volume.²⁰ Calcium glucubionate is still commonly used, however, especially for treatment of MBDs in reptiles, particularly because of safety. I select and prefer using human products with calcium carbonate. Unfortunately, nutraceutical products for animals, such as vitamin and mineral supplements, are not heavily regulated and there are significant concerns based on published studies as to what certain animal products report to contain and what they actually contain, whether or not there is batch variation or even if every sample is tested for quality control. My opinion is that there is no purpose in supplementing any human or pet product for a herptile containing phosphorus without evidence of a phosphorus deficiency. High percentages of calcium in the diet can affect palatability to certain herptiles. Calcium sand as substrate has been found to cause impactions in herptiles and is not recommended to use as a substrate or as a calcium supplement.

As discussed previously, oral cholecalciferol supplementation is necessary in herptiles not entirely reliant on UV radiation for vitamin D₃ creation. Yet, how to do this and deciding which products to use present a conundrum. Does dusting provide much nutrient benefit or is it a wasted effort altogether? Use a product with added calcium? What is benefit to having phosphorus in the mixture? Should human products be used instead of pet products because of the higher standards they must meet (albeit fewer standards than for medications)? For some species, an ideal situation seems to be using a combination of UV radiation and oral cholecalciferol. How much is needed and how much is too much? Should it be gut loaded into the invertebrates' gastrointestinal system or applied topically? These are all questions that need scientific, evidence-based answers and until then, we are often left guessing or extrapolating. Hemolymph collected from six wild-caught, subadult goliath birdeater spiders (*Theraphosa blondi*) was analyzed for calcidiol and was detected in all of the spiders with a mean of 5.7 nmol/L (± 1.5 nmol/L). How they got calcidiol was unknown; UV radiation was thought unlikely and dietary from prey invertebrates was thought possible.²¹

Gut loading and dusting of invertebrates has been recommended for many years to compensate for their naturally poor calcium content (exceptions are snails and earthworms [*Lumbricus terrestris*] fed calcium-rich soil). Gut loading refers to feeding the invertebrate a calcium-rich diet, then feeding the invertebrate to provide calcium to the herptile. Diets too high in calcium, however, are considered unpalatable in many crickets (*Acheta domesticus*). Dusting (or shake and bake) invertebrates may provide some extra calcium; however, toxicity reports in certain anurans and the rapid loss of the calcium dust from the invertebrate, should it not be eaten immediately, need to be considered.²² Some invertebrates, such as wax worms (*Galleria Mellonella*) and Phoenix worms (*Hermetia illucens*) are not able to be gut loaded, so any supplemental calcium or cholecalciferol could be at best dusted on them. Phoenix worms, the larval form of the soldier fly, may have a moderately good calcium/phosphorus ratio, but there have been anecdotal reports by my colleagues of unpalatability, undigestibility, and even potential gastrointestinal rupture. This can vary by species of herptile fed to, but caution and further research are recommended. A recent study found that feeding of Mazuri Hi-Ca Cricket diet (<http://www.mazuri.com/PDF/5m38.pdf>) to mealworms (*Tenebrio molitor*) and superworms (*Zophobas morio*) enabled the nutrient composition of the larvae to reach a calcium/phosphorus ratio of 1.31:1 in mealworms (or 2.42 g/kcal) and 1.47:1 in superworms after 48 hours of feeding on the diet. These ratios are reported to be slightly lower than those recommended for rats (1.66:1); however, there are no nutritional evaluations for the needs of any herptile.²³ Two studies looked at various diets fed to crickets and found that certain calcium-fortified diets increased the crickets' calcium levels compared with controls but found several of the diets advertised as calcium fortified did no better than an unfortified control diet.^{24,25} Donoghue²² has an excellent chart in her chapter in *Reptile Medicine and Surgery* that shows the natural, unsupplemented calcium and phosphorus levels of many commonly fed invertebrates. Earthworms were the only ones with a better than a 1:1 calcium/phosphorus ratio, with most other ratios approaching an inverted 1:6 versus the recommended 2:1 or greater. Most vertebrates have an adequate ratio of calcium to phosphorus, although immature specimens, such as 1-day-old chickens (*Gallus domesticus*) or pinkie mice (*Mus musculus*), are considered inadequate for long-term calcium sources.

For herbivores, the key portion of the diet from a calcium perspective is lots of leafy greens. These are often best purchased in a prepackaged salad mix, with the spring mix best in my opinion. Many older references recommend other greens, such as kale or chard, but these greens have strong flavors and can be rejected by herbivorous reptiles. Noniceberg lettuces have great calcium/phosphorus ratios, sprouts tend to have inverted ratios, and most other vegetables have at best a 1:1 ratio, but many are inverted. With fruits, most are inverted ratios; however, berries often are 1:1. Prickly pear cactus and alfalfa hay have excellent ratios, with timothy hay less so and orchard grass having an inverted one. Oxalates are a compound found in some plants that bind calcium. Spinach, rhubarb, cabbage, peas, potatoes, and beet greens contain these and should be avoided in MBD reptiles.²²

A concern with aquatic species, such as semiaquatic chelonians, semiaquatic lizards and snakes, and amphibians, is the effect of calcium in the environmental water. Distilled and demineralized water have all minerals, including calcium salts, removed. These should particularly be avoided with amphibians. Softened water has sodium replacing calcium and magnesium. Calcium-enhanced water leads to significantly greater absorption of calcium in humans when compared with dairy products; however, this has not been studied in herptiles.²² With RSHPT, ideal species hydration is obvious. Many herptiles not only drink water but also engage in cloacal

drinking, passive absorption of the water into the cloaca for storage in urinary bladders, the cloaca itself, or even ureters in those species lacking a urinary bladder. I recommend mandatory soaks 1 to 7 times weekly for many herptiles, depending on their hematocrit and plasma protein levels, owner's geographic location, and environmental conditions of the immediate enclosure. Many arboreal species are ill suited for soaking, and many desert species may not handle even slightly deep water well. I recommend reptiles be soaked in a separate container, at a depth of one-quarter the height of the animal's body at approximately 43°C (110°F). Amphibians differ by a recommended temperature of no greater than 27°C (82°F). Usually amphibians' environments are very humid, so this recommendation generally pertains more to terrestrial salamanders and anurans, in particular toads. In most cases, the water cools significantly within 10 to 15 minutes, so the water should be refreshed or the process completed. If there is concern with a specific individual animal, put the container at a slight tilt so the deep end meets the one-quarter height requirement and the shallow end allows the head to be free of water. Many substrates used in cages (chips, barks, and recycled newspaper pellets) are by their nature dehydrating and can contribute to an animal's subclinical chronic dehydration, particularly in nondesert species.

A side note is a condition in chelonians known as pyramiding. The carapaces of captive-raised tortoises often develop a pyramid-shaped osseous growth centrally within the horny plates. With few exceptions (Indian star tortoises [*Geochelone elegans*]), this conical growth pattern is considered pathologic. Fifty recently hatched African spurred tortoises (*G. sulcata*) were raised for 5 months under artificial conditions of varying environmental humidity and dietary protein content (14% vs 19% vs 30% crude protein in dry matter). Dry environmental conditions (24.3%–57.8% and 30.6%–74.8% relative humidity) produced taller humps than humid conditions (45%–99% relative humidity). Hump formation differed significantly between these three groups kept under different humidity conditions. Variable dietary protein had a minor, positive impact on this pathologic formation of humps. Levels of calcium and phosphorus in the blood led to no further explanation as to the development of the humps.^{26,27} More studies needed to further elucidate complete etiology behind this condition and perhaps calcium metabolism may play a role.

A final environment-related note for herptiles with MBDs is consideration of limiting opportunities for animals with potentially extremely brittle bones to fall or otherwise injure themselves. Removal of climbable items is recommended for many herptiles that can adapt well to a single level environment; however, species, such as chameleons, may require instead cushioning below needed branches, using uncompacted and hydrated sphagnum moss or even old pillows.

TREATMENT

Once an MBD is diagnosed, treatment options can be selected but should be based in many cases on total calcium, ionized calcium, and other diagnostic test results. Previous sections of this article have focused on recommendations for developing a normal environment and diet, and often implementing these recommendations is where efforts are best directed, from an energy and financial perspective. There are some medical therapeutics, however, that may enhance the recovery of these patients; however, there are few, if any, studies in herptiles documenting their effectiveness, so most recommendations are based on anecdotal evidence or extrapolation from human or mammalian medicine. Intravenous (or intramuscular [IM] or subcutaneous) calcium is often considered for MBDs; however, its use is controversial and can be fatal. Very low total calcium in certain mature herptiles has been

responsive to IV calcium in a handful of my cases but is usually only given to seizing animals or those that are comatose and with requisite low calcium values as a last resort. I have had only 5 cases in 13 years respond out of approximately 25 attempts. Giving calcium IM or subcutaneously seems to have little benefit and is painful, so extremely aggressive intervention (IV) should be performed or, due to the chronicity of these cases, oral supplementation is generally the best approach. Other practitioners have had some success, however, in treating hypocalcemic tetany with IM or intracoelomic calcium.³

Salmon calcitonin has been used by many practitioners, including me, to manage certain cases of MBDs. It is critical to not administer this if an animal has low blood calcium so is often provided once a patient is stabilized. In humans, salmon calcitonin provides an analgesic effect for the pain usually found with MBDs. In monitoring salmon calcitonin's benefits for patients by monitoring calcium levels, it must be remembered that it only has an effect on ionized calcium levels.³ Calcitriol supplementation has been discussed previously and is often necessary; however, parenteral administration of a vitamin D product should be carefully considered and is generally not recommended. Bisphosphonates are a class of drugs used in humans to prevent the loss of bone mass and to treat osteoporosis, osteitis deformans, bone metastasis (with or without hypercalcaemia), multiple myeloma, primary hyperparathyroidism, osteogenesis imperfecta, and other conditions that feature bone fragility. Bisphosphonates inhibit the digestion of bone by osteoclasts by encouraging osteoclasts to undergo apoptosis. These may be a future area of research for potential treatment in herptiles with MBDs.

Never underemphasize the necessity of fluids for the MBD-afflicted patients. Fluids can be administered in many different ways, from IV/interosseously to oral to cloacal drinking, but whether or not the diagnosis is RSHPT or some other type, rehydration must be addressed. Nutritional support of a balanced diet in the critical phases is addressed by me by using the carnivore or herbivore critical care diets from Oxbow Animal Health (Murdock, Nebraska; <http://www.oxbowhay.com>).

In some cases, these herptiles may have suffered fractured bones. Often these fractures healed quickly, albeit via malunion. With spinal fractures, prognoses are often guarded, although some regain hind limb function once swelling and remodeling have resolved. In the short term, an honest discussion of quality of life needs to occur with owners, and regular enemas to help express feces and urine are required, if an animal is unable to urinate or defecate on its own. With limb fractures, surgical repair (internal and external) should be avoided whenever possible, because the likelihood of anesthetic recovery is lower in MBD animals and the likelihood of iatrogenic additional fractures is higher. Splinting limbs to the body or tail or using smoothed syringe cases as splints has worked for me in the past. Pain in these patients with MBDs, whether or not fracture, is described as considerable in some humans and mammals and should be assumed to be so in herptiles until proved otherwise. Management of pain is in its infancy in terms of understanding in herptiles, so guidance on ways to manage this are best left for future articles on pain management.

REFERENCES

1. Available at: <http://www.uvguide.co.uk/whatreptilesneed.htm>. Accessed January 16, 2010.
2. Antwis RE, Browne RK. Ultraviolet radiation and vitamin D-3 in amphibian health, behaviour, diet and conservation. *Comp Biochem Physiol A Mol Integr Physiol* 2009;154(2):184–90.

3. Mader D. Metabolic bone disorders. In: Mader D, editor. Reptile medicine and surgery. 2nd edition. St. Louis (MO): Saunders/Elsevier; 2006. p. 841–51.
4. Wright K. Two common disorders of captive bearded dragons (*Pogona vitticeps*): nutritional secondary hyperparathyroidism and constipation. Journal of Exotic Pet Medicine 2008;17(4):267–72. Available at: http://www.exoticpetmedicine.com/issues/contents?issue_key=S1557-5063%2808%29X0005-9. Accessed May 4, 2010.
5. Isaza R, Garner M, Jacobson E. Proliferative osteoarthritis and osteoarthritis in 15 snakes. J Zoo Wildl Med 2000;31(1):20–7.
6. Dennis PM, Bennett RA, Harr KE, et al. Plasma concentration of ionized calcium in healthy iguanas. J Am Vet Med Assoc 2001;219(3):326–8.
7. Acierno MJ, Mitchell MA, Roundtree MK, et al. Effects of ultraviolet radiation on 25-hydroxyvitamin D3 synthesis in red-eared slider turtles (*Trachemys scripta elegans*). Am J Vet Res 2006;67(12):2046–9.
8. Eatwell K. Plasma concentrations of 25-hydroxycholecalciferol in 22 captive tortoises (*Testudo* species). Vet Rec 2008;162(11):342–5.
9. Ferguson GW, Gehrmann WH, Karsten KB, et al. Do panther chameleons bask to regulate endogenous vitamin D3 production? Physiol Biochem Zool 2003;76(1):52–9.
10. Laing CJ, Trube A, Shea GM, et al. The requirement for natural sunlight to prevent vitamin D deficiency in iguanian lizards. J Zoo Wildl Med 2001;32(3):342–8.
11. Ramer JC, Maria R, Reichard T, et al. Vitamin D status of wild Ricord's iguanas (*Cyclura ricordii*) and captive and wild rhinoceros iguanas (*Cyclura cornuta cornuta*) in the Dominican Republic. J Zoo Wildl Med 2005;36(2):188–91.
12. Zotti A, Selleri P, Carnier P, et al. Relationship between metabolic bone disease and bone mineral density measured by dual-energy X-ray absorptiometry in the green iguana (*Iguana iguana*). Vet Radiol Ultrasound 2004;45(1):10–6.
13. Hernandez-Divers SJ, Stahl SJ, Stedman NL, et al. Renal evaluation in the healthy green iguana (*Iguana iguana*): assessment of plasma biochemistry, glomerular filtration rate, and endoscopic biopsy. J Zoo Wildl Med 2005;36(2):155–68.
14. Greer LL, Daniel GB, Shearn-Bochsler VI, et al. Evaluation of the use of technetium Tc 99m diethylenetriamine pentaacetic acid and technetium Tc 99m dimer-captosuccinic acid for scintigraphic imaging of the kidneys in green iguanas (*Iguana iguana*). Am J Vet Res 2005;66:87–92.
15. Sykes JM 4th, Schumacher J, Avenell J, et al. Preliminary evaluation of 99mTechnetium diethylenetriamine pentaacetic acid, 99mTechnetium dimercaptosuccinic acid, and 99mTechnetium mercaptoacetyltriglycine for renal scintigraphy in corn snakes (*Elaphe guttata guttata*). Vet Radiol Ultrasound 2006;47(2):222–7.
16. Ferguson GW, Gehrmann WH, Karsten KB, et al. Ultraviolet exposure and vitamin D synthesis in a sun-dwelling and a shade-dwelling species of *Anolis*: are there adaptations for lower ultraviolet B and dietary vitamin D3 availability in the shade? Physiol Biochem Zool 2005;78(2):193–200.
17. Karsten KB, Ferguson GW, Chen TC, et al. Panther chameleons, *Furcifer pardalis*, behaviorally regulate optimal exposure to UV depending on dietary vitamin D3 status. Physiol Biochem Zool 2009;82(3):218–25.
18. Mitchell M, Thompson D, Augustine R, et al. Effects of ultraviolet radiation on 25-hydroxyvitmain D3 synthesis in juvenile Blanding's turtles (*Emydoidea blandingii*). Proceedings of the Annual Conference of the Association of Reptilian and Amphibian Veterinarians. Milwaukee (WI): Omnipress; 2009. p. 137.
19. Fledelius B, Jørgensen GW, Jensen HE, et al. Influence of the calcium content of the diet offered to leopard tortoises (*Geochelone pardalis*). Vet Rec 2005;156(26):831–5.

20. Available at: <http://en.wikipedia.org/wiki/Calcium>. Accessed January 19, 2010.
21. Zachariah TT, Mitchell MA. Vitamin D₃ in the hemolymph of goliath birdeater spiders (*Theraphosa blondi*). J Zoo Wildl Med 2009;40(2):344–6.
22. Donoghue S. Nutrition. In: Mader D, editor. Reptile medicine and surgery. 2nd edition. St. Louis (MO): Saunders/Elsevier; 2006. p. 251–98.
23. Latney L, Toddes B, Wyre N, et al. Evaluation of the nutrient composition of *Tenebrio molitor* and *Zophobas morio* fed supplemental diets utilized to improve the nutrition of insectivorous animals. Proceedings of the Annual Conference of the Association of Reptilian and Amphibian Veterinarians. Milwaukee (WI): Omnipress; 2009. p. 173.
24. Finke M, Dunham S, Kwabi C. Evaluation of four dry commercial gut loading products for improving the calcium content of crickets, *Acheta domesticus*. J Herp Med Surg 2005;15(1):7–12.
25. Finke M, Dunham S, Cole J. Evaluation of various calcium-fortified high moisture commercial products for improving the calcium content of crickets, *Acheta domesticus*. J Herp Med Surg 2004;14(2):17–20.
26. Wiesner CS, Iben C. Influence of environmental humidity and dietary protein on pyramidal growth of carapaces in African spurred tortoises (*Geochelone sulcata*). J Anim Physiol Anim Nutr (Berl) 2003;87:66–74.
27. Available at: <http://en.wikipedia.org/wiki/Bisphosphonate>. Accessed January 22, 2010.